INTERACTION OF EXPOSURE CONCENTRATION AND DURATION IN DETERMINING ACUTE TOXIC EFFECTS OF SARIN VAPOR IN RATS

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ABSTRACT

These studies were conducted as part of the "Low Level Operational Toxicology" program which began by addressing the question of how low do chemical agent detectors need to go to measure toxicologically significant effects of the classical chemical warfare (CW) agents. Initial phases of the program have focused on GB vapor. The objective of these studies was to 1) identify threshold exposure conditions at which toxicologically significant effects occur in the rat and 2) develop models for predicting dose-response effects of low CW agent concentrations as a function of exposure duration. Sarin (GB) vapor exposure is associated with both systemic and local toxic effects occurring primarily via the inhalation and ocular routes. These studies examined the effects of varying exposure concentration and duration on the probability of lethality occurring in rats exposed to Sarin (GB) vapor. Groups of male and female rats (Sprague-Dawley) were exposed to one of a series of GB vapor concentrations for a single duration (5- 360 minutes) in a whole-body dynamic chamber. The onset of clinical signs and changes in blood cholinesterase activity were measured with each exposure. Separate effective concentrations for lethality (LC50) and miosis (EC50) in 50% of the exposed population and corresponding dose-response slopes were determined for each exposure (duration) by the Bliss probit method. A predictive model derived from multifactor probit analysis describing the relationship between exposure conditions and probability of lethality in the rat is presented. Contrary to that predicted by Haber's rule, LCt_{50} values increased with exposure duration (i.e., the CT for 50% lethality exposed population and corresponding dose-response slope was not constant over time). A plot of Log (LCt50) versus Log (Exposure Time) showed significant curvature. To account for this curvature, an interactive term, Log (C)*Log(T), was added to the toxic load model. Overall, female rats were more sensitive to GB vapor toxicity than male rats over the range of exposure concentration and duration studied. Miosis was the initial clinical sign noted following the start of GB vapor exposure. Although blood cholinesterase activity was significantly inhibited by GB vapor exposure, poor correlation between cholinesterase inhibition and exposure conditions or cholinesterase inhibition and severity of clinical signs was noted

INTRODUCTION

Acute whole-body exposure to Sarin (GB) vapor results in both systemic and local toxic effects, which are primarily mediated via inhalation and ocular routes, respectively. In order to assess the acute health hazards of such an exposure, the probability of GB-induced biological effects must be quantitatively related to exposure parameters, including both atmospheric concentration (C) and exposure time (duration) (T).

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Historically, it has been standard practice to use a linear time-integrated concentration (i.e., C x T, CT, or dosage) to predict mortality-response relationships for chemical vapor exposures. The above concept has persisted as an accepted principle in military hazard assessment, currently serving as the basis for estimating injury from exposure to chemical warfare (CW) agents. This measure of exposure was first attributed to Haber (1924), who found that for certain poison gases (e.g. phosgene) used in the First World War, the toxic effects appeared to be correlated with the dosage (at least between 5 min and 8 hr durations). Haber's law or rule, as commonly understood in inhalation toxicology, states:

$$C \times T = k \qquad [1]$$

with regard to the incidence of a particular biological effect. A linear time-integrated concentration implies concentration and duration are equally important components in quantifying the toxic potential of an exposure.

Alternatively, ten Berge et al., (1986) demonstrated that:

$$C^n T = k \qquad [2]$$

correlates well with the degree of injury; where *C* is the (constant) concentration, *T* is the exposure time, and *n* is an index (the so-called *toxic load* exponent) that depends on the particular gas or aerosol, and exposure scenario. In general, for most gases for which experiments have been conducted, *n* was found greater than one. In order to determine the value for *n*, an experiment must be designed such that both exposure concentration and duration are varied in the same study. If the above concepts for quantifying health risks of toxic gases are also true for chemical nerve agents, the traditional use of "dosage" (i.e., C x T) in estimating casualty/risk is not appropriate. Military deployment operations, or handling, storage, and destruction of chemical agents as well as emergency response procedures will require the most accurate and up-to-date assessment of agent-related health hazards.

A major objective of the present study was to determine the relationship between GB vapor exposure concentration (C), duration of exposure (T) and the probability of a toxic effect (lethality). Prior to this study, the range of exposure times cited in the literature for mammals was limited to relatively short acute exposures (from seconds to several minutes) (Yee, 1996). The present study examined acute exposure times from 5 to and including 360 min, for which little data has been published. Data generated from this study were used in formulating concentration-exposure time-response models for more accurately estimating the probability of lethality given a combination of exposure concentration and duration. Future studies will extend this approach to characterize responses to other nerve agents for which data gaps exist, biological responses to very low concentrations of chemical nerve agents, as well as differences among mammalian species.

MATERIALS AND METHODS

GB Vapor Generation. Sarin (GB) samples (97.2 ± 0.2% purity by NMR ^{31}P) were obtained from the US Army Egewood Chemical Biological Center. A modified spray atomization system produced GB droplets (<15μ) that quickly evaporated into vapor which was drawn through a 750-liter dynamic air-flow inhalation chamber constructed of stainless steel with Plexiglas windows.

Vapor Sampling/Anaysis. Three methods were used to sample/monitor and analyze GB vapor concentration in exposure chamber: a) *"Edgewood" bubblers* (containing hexane)/ gas chromatograph with flame photometric detection (GC-FPD) b) *solid sorbent tubes* (Tenax-TA)/gas chromatograph with flame ionization detection (GC-FID) and c) a *phosphorus monitor* (HYFED, Model PH262) provided a

continuous strip chart record of rise, equilibrium, and decay of the chamber vapor concentration during an exposure.

Animal Exposures and Measures of Clinical Response. Sprague-Dawley rats (8-10 weeks old from Charles River Laboratories) were confined in stainless steel compartmentalized cages (20" x 14" x 4") with each rat free to move within a separate compartment. Male (10 rats/exposure group/concentration) and female (10 rats/exposure group/concentration) rats were exposed (whole body, dynamic mode) to a fixed concentration of GB vapor for a fixed duration. Rats were exposed to one of five concentrations (2 – 56 mg/m^3) of GB vapor for one of seven exposure times (5, 10, 30, 60, 90, 240 or 360 min). Lethality and sub-lethal clinical signs (e.g., miosis, tremors, salivation, labored breathing and convulsions) were monitored during and after exposure. Lethality was counted as the fraction of each exposure group which died within 14 days after exposure. Blood cholinesterase (AChE and BuChE) activity was measured (Ellman, 1961) from pre and post-exposure blood samples collected from a tail vein. The effects of GB vapor exposure on pupil size (diameter) were assessed pre- and post-exposure under a 100 ft candle light source using a simple microscope (Bausch & Lomb, 20x) with a reticule eyepiece insert.

Data Analysis. A probit analysis program (MINITAB®, Version 13) was used to generate a separate dose-response curve (with slope, intercept and 95% fiducial limits) for each duration of exposure tested and to determine if gender differences exist in the sensitivity to the toxic effects of GB vapor exposure. Binary normal regression (multifactor probit analysis) was used to model the relative effects of exposure concentration and duration on probability of lethality or miosis. Differences in pre-exposure vs. post-exposure cholinesterase levels were expressed as a percent change resulting from treatment. This graded response was plotted against CT using linear regression in order to determine if correlations exist as indicated by significant regression coefficients.

RESULTS

LC_{50}, LCT_{50}, 95% fiducial limits, and slopes are listed for each exposure duration in Table 1. Plots of LC50 vs time are shown in Figure 1. In the first phase of this study, exposure conditions were optimized for estimating LC_{50} and LCt_{50}. The study design was not optimized for estimating EC_{50}s for sublethal signs.

Blood AChE and BuChE activities (expressed as percent of pretreatment) were inhibited as a result of exposure to various combinations of GB vapor concentration and time. Most responses (for 10, 30, 90 and 240 min exposures) appeared to fall between 5 and 30% of pretreatment baseline (Figure 2). AChE and BuChE activity (percent of pretreatment) was poorly correlated with exposure conditions (CT) in the lethal range of exposures. Median pretreatment levels of BuChE activity were consistently higher ($P< 0.001$) in female (1750 U/ml) than in male rats (424 U/ml), as determined by the Mann-Whitney Rank Sum test. However, no differences were noted between pretreatment male and female AChE activity.

All combinations of GB vapor exposure concentration and time resulted in complete miosis (pinpoint pupil) in male and female (Figure 3) rats as measured at the first hour post-exposure. This was followed by a transient mydriasis (dilated pupil) between 24-48 h post-exposure ($p < 0.01$) lasting several days. At 7 days post-exposure pupil diameters were decreasing in size ($p <0.01$) but still greater ($p <0.01$) than pre-exposure sizes.

DISCUSSION

Predicting lethality with exposure concentration models. Adequacy of exposure concentration models (Haber's rule and "toxic load") for predicting GB vapor-induced lethality were tested by regressing log(LCT50) on log(Time) and [log(Time)] squared. The statistical significance of the squared term in log (Time) shows that the plot of log(LCT50) versus log(Time) (Figure 1) is not a straight line with slope other than -1 (as predicted by the toxic load model) nor a straight line with a slope of –1 (Haber's rule) but instead a curved line. Given that the resulting squared term was statistically significant, there was adequate evidence, from a statistical point of view, to reject both Haber's rule and the toxic load model.

Starting with 12 terms [constant, C, T, C^2, T^2, C*T, Sex, Sex*C, Sex*T, Sex*C^2, Sex*T^2, and Sex*C*T, where C = centered log(Conc), T = centered log(Time), C^2 = C*C, etc., and Sex = 1 (male) and –1 (female)], the least significant term (largest p value) was deleted followed by reanalysis. This process was reiterated until all terms were significant ($p < 0.05$) in order to reduce the multicollinearity in the model. The model produced from this process is described below. The backwards elimination procedure described above resulted in the following significant terms (Table 2):

TABLE 1. Summary of LC50, LCT50, slopes and fiducial limits for GB Vapor-induced lethality (14 days post exposure) at each of seven exposure durations.

Exposure Duration (min)	Sex	LC50 (mg /m3)	95% F.I.	Slope	Sex	LC50 (mg /m3)	95% F.I.	Slope
5	F	32.8	29.8 –36.6	10.3	M	45.9	40.0 – 51.3	9.4
10	F	18.1	16.3 - 20.4	11.9	M	22.6	20.8 – 24.8	16.4
30	F	8.51	7.79 - 9.31	12.9	M	8.84	8.20 - 9.47	21.6
60	F	6.39	5.72 – 6.95	13.0	M	7.55	*	6.1
90	F	4.46	4.24 - 4.69	22.1	M	4.81	4.58 - 5.12	21.5
240	F	3.03	2.66 - 3.37	9.9	M	4.09	3.66 - 5.00	8.0
360	F	2.63	2.44 - 2.82	14.9	M	2.89	2.69 - 3.15	13.2
Exposure Duration (min)	Sex	LCt50 (mg. min/m^3)	95% F.I.	Slope	Sex	LCt50 (mg. min/m^3)	95% F.I.	Slope
5	F	164	149 -183	10.3	M	230	200 - 257	9.4
10	F	181	163 - 204	11.9	M	226	208 - 248	16.4
30	F	255	234 - 279	12.9	M	265	246 - 284	21.6
60	F	383	343 - 417	13.0	M	453	*	6.1
90	F	401	382 - 422	22.1	M	433	412 - 461	21.5
240	F	727	638 - 809	9.8	M	982	878 - 1200	8.0
360	F	947	878- 1015	14.9	M	1040	968 - 1134	13.2

*-Not able to calculate due to non-significant slope at 95% level.

TABLE 2. Summary of Binary Normal Regression Fitted Coefficients, Associated Errors and Statistical Significance for the Interaction Model [3].

Predictor	Coef.	S.E. Coef.	z	p
Constant	0.669	0.1686	3.97	0.000
Sex	-0.4238	0.0934	-4.53	0.000
cLogC	11.171	1.5000	7.45	0.000
cLogT	6.7223	0.9097	7.39	0.000
cLogCcLogT	1.8936	0.4156	4.56	0.000
Sex*cLogC	-0.3813	0.1783	-2.14	0.033

Thus, for probability of lethality let Y= normit (Probit -5):

$$Y = 0.6691 - 0.42381*Sex + 11.171*cLogC + 6.7223*cLogT + 1.8936*cLogC*cLogT - 0.3813*Sex*cLogC; \quad [3]$$

for males, this reduces to

$$Y = 0.2453 + 10.7897*cLogC + 6.7223*cLogT + 1.8936*cLogC*cLogT, \quad [4]$$

and for females, to

$$Y = 1.0929 + 11.5523*cLogC + 6.7223*cLogT + 1.8936*cLogC*cLogT. \quad [5]$$

$$cLog(X) = Log\ X - Mean\ Log\ (X);\ where\ X = C\ or\ T \quad [6]$$

Means for centering: Log(C) = 0.951702; Log(T) = 1.67781.

Predicted LCT_{50} values from Equations 4 and 5 are plotted in Figure 4. Equations 4 and 5 are an extension of the toxic load model. They are referred to here as the "interaction" model because of the presence of the interaction term, clogC*clogT.

Male vs. Female Sensitivity to GB Vapor-induced Lethality. Female rats were more sensitive to the lethal effects of GB vapor than males in the present study based on the significance of the Sex term in Equation 3 ($p<0.001$). In addition, a review of the clinical sign data suggests that clinical signs of toxicity appeared earliest in females, as a group, and progressed to more severe levels than in male rats. These findings are consistent with those of Callaway and Blackburn (1954) (for 1 min exposures) who reported that female rats were almost twice as sensitive to the lethal effects of GB vapor than males. McPhail (1953) reported that the male mouse was more sensitive to GB vapor than the female but the reverse was true for the hamster and the rat. In addition, female rats have been shown to be more sensitive than males to the lethal effects of Soman (Sket, 1993). In female rats, the LD50 for Soman was only about half that of males. This pattern was also reported for lethal exposure of rats to some organophosphate insecticides (Sheets et al., 1997).

GB Vapor Effects on Blood AChE and BuChE.. The most commonly accepted mechanism by which nerve agents are believed to induce acute toxicity is by inhibition of AChE activity in target tissues. Although red blood cell (RBC) and plasma cholinesterase activities are routinely monitored as a sensitive index of exposure to anti-cholinesterase agents, they by no means imply anti-cholinesterase intoxication (Koelle, 1994). In a review of theories and therapy of intoxication by potent AChE compounds, Ellin (1981) suggested that poor correlation exits between the clinical picture of poisoning by OP compounds and levels of ChE activity. According to Ellin et al. (1981), solutions to this problem might be obtained in pharmacodynamic studies which relate to binding of OP compounds to ChE and other esterases in tissue and blood as well as their rates of elimination. Symptoms and treatment of patients accidentally

exposed to ChE inhibitors, Sarin and Soman, are discussed by Sidell (1992). He suggests that activity of the circulating ChE does not parallel the activity of ChE in tissue and that tissue function can be reasonably normal even with minimal ChE activity. If an OP compound is administered in low concentration levels over a long period, blood levels of an animal can drop to near zero, yet the animal survives. This was observed in the present study (Figure 2). If blood levels are caused to drop to zero rapidly, the animal dies.

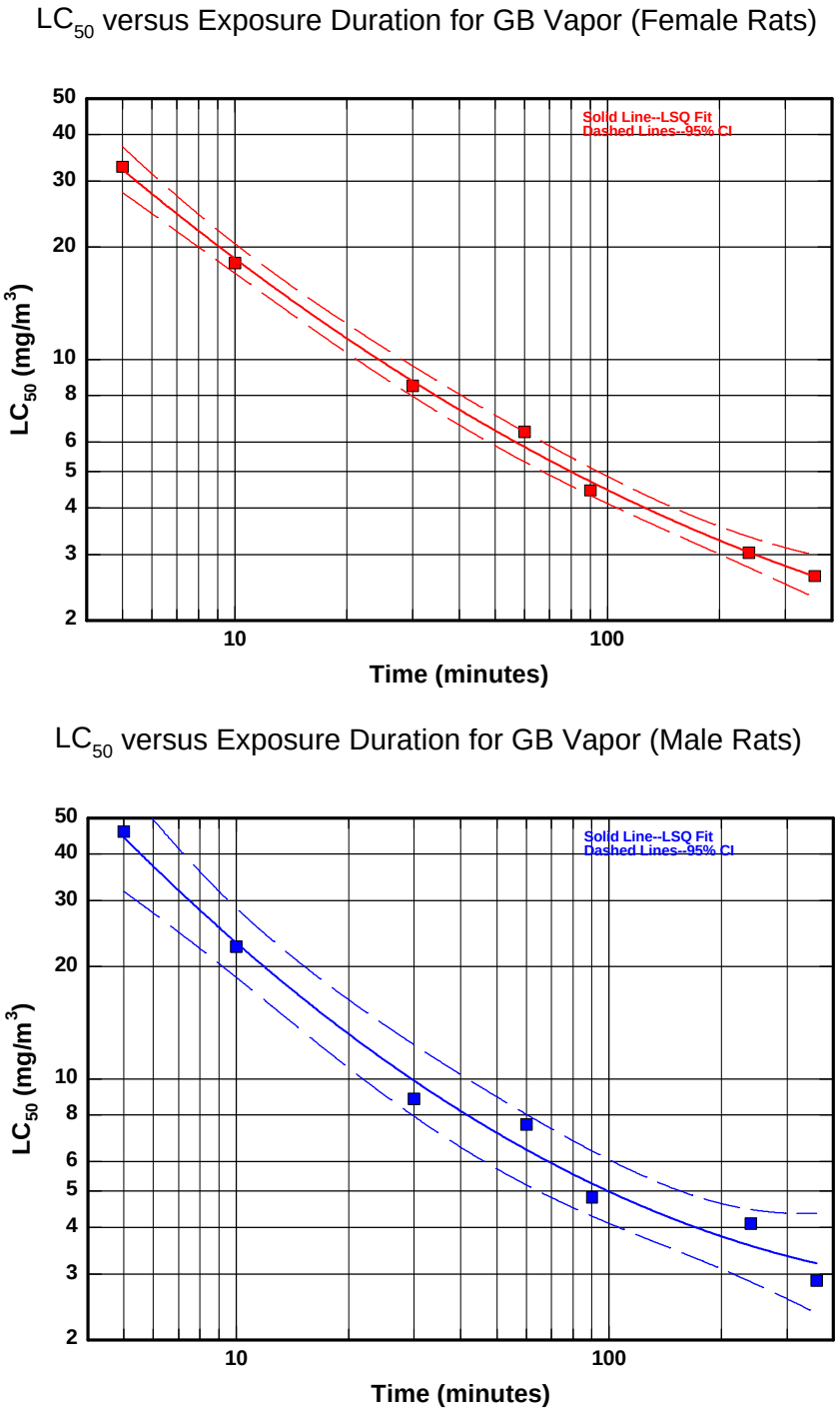

Figure 1. LC_{50} vs. Duration (minutes) of GB Vapor Exposure in Rats.

Pupil Responses to GB Vapor Exposure. Given that the exposure concentrations tested in this study were selected to optimize LC50 responses, it is not surprising that maximal miosis was seen in all exposed rats over the first 48-hrs post exposure. However, the marked and consistent reversal of this response progressing to a limited duration of mydriasis was not expected. Such responses are rarely, if ever, reported in the literature. Perhaps pupil effects were seldom monitored beyond the onset of miosis and the return to a pupil diameter within the pretreatment range. GB vapor-induced changes in pupil size of the present study are likely a local effect of GB on the eye. Presumably, GB exposure altered the balance between sympathetic vs. parasympathetic control over the pupil size, which changed over time following exposure. In proposing an explanation, it can be speculated that sometime during and following (within 48 hrs) exposure, the cholinergic component would likely predominate in the absence of AChE activity, resulting in miosis. At longer periods (4-6 days post exposure) cholinergic input would be desensitized thus leaving the noradrenergic component with predominant control resulting in mydriasis until the opposing mechanisms return to normal function and the balance is restored

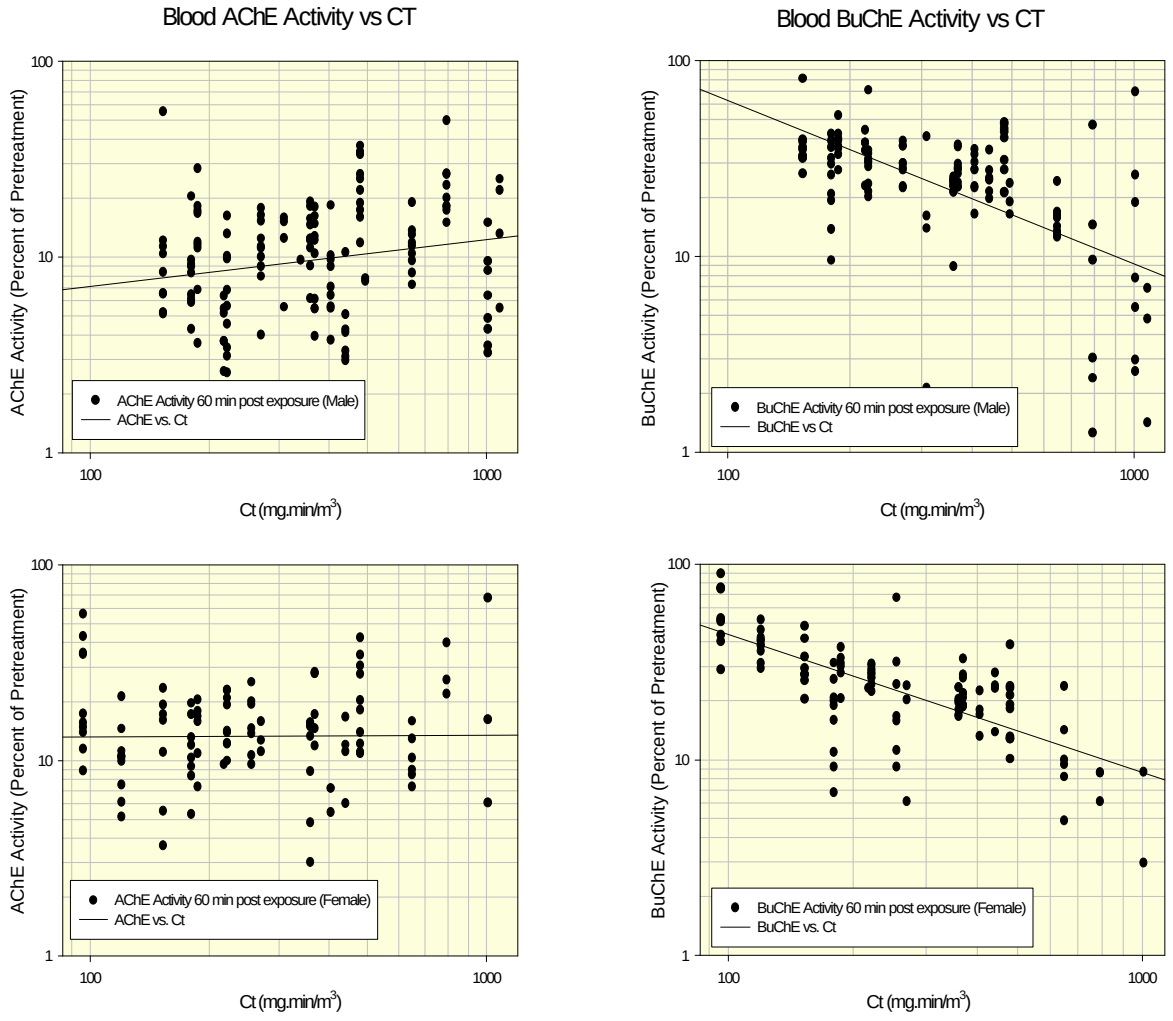

Figure 2. Blood AChE and BuChe (percent of pretreatment activity) vs. CT in GB vapor exposure (for 10, 30, 90 and 240 min exposures).

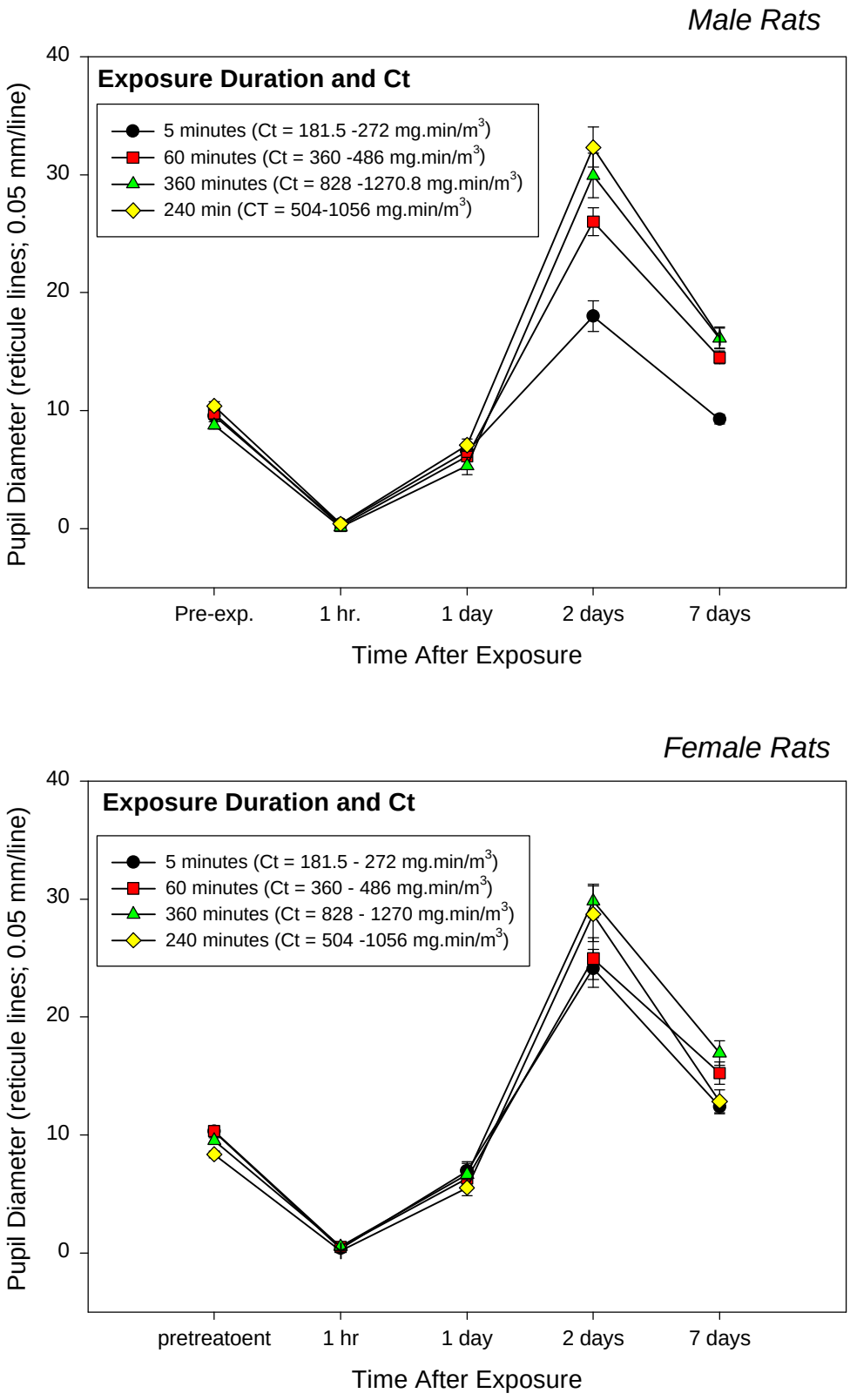

Figure 3. Effects of GB Vapor Exposure (240 min) on Pupil Diameter.

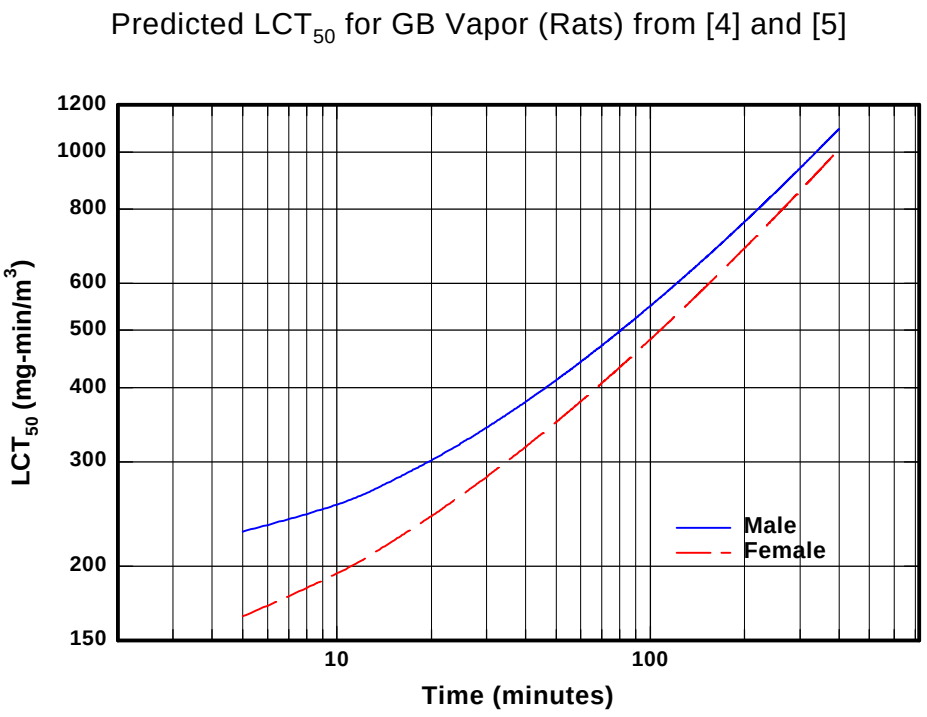

Figure 4. Predicted LCT_{50} versus Exposure Time.

CONCLUSIONS

This study examined the relationship between exposure concentration-time and lethal response in rats exposed to GB vapor. It was found that neither Haber's rule nor the toxic load model adequately explain the relationship between exposure conditions and probability of lethal response in the rat. An analysis of the randomized Part-II data led to the interaction model Y = b0 + b1* Log(C) + b2* Log (T) + b3* Log (C)*Log(T) as an extension of the toxic load model. Overall, female rats appeared to be more sensitive to GB vapor toxicity than male rats over the concentration and time range studied. Various sub-lethal clinical signs (miosis, salivation, tremors, convulsions, and blood cholinesterase activity) were also observed, but since the exposure conditions were optimized for lethality, effective concentrations (e.g., ECt_{50}) could not be determined. Miosis was maximal at all combinations of GB vapor concentration-time studied, and appeared to be the most sensitive clinical sign of GB exposure recorded. Miosis briefly progressed to mydriasis before returning to pupil sizes closer to the normal range. Although GB vapor exposure concentration (Ct) was correlated with the inhibition of blood cholinesterase activity, this correlation can not be used for estimating the level nor clinical severity of GB vapor exposure.

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